REMARKS

The rejections of Claims 9, 15, 21 and 27 under 35 U.S.C. § 102(b) as anticipated by, and of Claims 10-14, 16-20, 22-26 and 28-32 under 35 U.S.C. § 103(a) as unpatentable over, US 5,240,900 (Nitsche), is respectfully traversed.

The presently-claimed invention is drawn to a method for alleviating a symptom from **lipopolysaccharide (LPS)-induced inflammation** comprising administering to a person orally or parenterally an effective amount of **human-type lactoferrin (hLf)** for a time and under conditions effective to alleviate said symptom (emphasis added). The symptom is accumulation of body fluid containing albumin at the inflammation site (Claim 9); accumulation of albumin at the inflammatory site (Claim 15); decrease of albumin concentration in blood (Claim 21); or increase of neutrophils in blood (Claim 27).

As described in the specification at paragraph (0003), lactoferrin (Lf) has been demonstrated in vitro to form a chelate with iron to mainly inhibit growth of E. coli, etc., and shows a bactericidal effect, as well as other pharmacological effects. While not particularly described in the specification, such other pharmacological effects are disclosed by Nitsche.

Beginning at paragraph (0004) of the specification, Applicants describe various aspects of inflammation and attempted solutions for inflammation and/or its symptoms. Thus, it is known that inflammation may be accompanied by, for example, accumulation of body fluid containing albumin or albumin *per se* at the inflammation site, decrease of albumin concentration in blood, or increase of neutrophils in blood. But none of the solutions in the prior art has disclosed or suggested the present use of hLf.

As will be more fully shown below, Nitsche is drawn to the use of Lf bound to a metal ion, not Lf *per se*, and is drawn to treating endotoxemia, and not a symptom from LPS-induced inflammation.

Nitsche begins by describing the original discovery of Lf and some of its known properties (column 1, line 10 through column 2, line 26). Nitsche then describes that endotoxin is a constituent of the cellular walls of gram-negative bacteria and is released only by the bacterial decay (column 2, lines 27-29). Endotoxin is transferred from the intestine into the blood, even in healthy persons, resulting in endotoxemia (column 2, line 66ff). The prior art has suggested various ways of reducing plasma endotoxin activity, such as by administering monoclonal lipid A antibodies, and other means, but with the exception of preparations containing monoclonal lipid A antibodies, all of the therapeutic means available to date are marred by the disadvantage that they inactivate only a certain percentage of the endotoxins entering the blood and thus, prove ineffective when larger amounts of endotoxins enter the bloodstream (column 3, lines 53-68). Nitsche addresses the problem, i.e., the search for a therapeutic agent suitable as a means for controlling the toxic effects of endotoxin by virtue of its ability to neutralize large amounts of plasma endotoxin rapidly, by the use of Lf that has bound a metal ion, which ion may be iron or another metal ion, wherein Lf used for parenteral applications must be equivalent to human Lf, while Lf from animal sources can be used in enteral applications (column 4, lines 3-25). Nitsche emphasizes that the Lf used **must** (emphasis added) have bound either iron or another metal ion (column 4, lines 15-17). Thus, the Examiner's finding that Nitsche discloses the administration of an effective amount of hLf or animal Lf **as an active agent for suppressing inflammation caused by endotoxin LSP-derived from gram-negative bacteria** (emphasis added), is clearly erroneous.

Nitsche does disclose various *in vitro* experiments in which human and bovine Lf were used in iron-free apoform (column 4, line 37ff). Among the results disclosed are that when endotoxin is incubated with iron-free apolactoferrin, an average reduction in endotoxin activity of 35.4 to 41.2% is registered, depending on the type and concentration of endotoxin (column 6, lines 17-20), although the results using Lf with bound metal ion are substantially

better (column 6, line 21ff). Even better results were obtained when the Lf bound to a metal ion is combined with an immunoglobulin (Ig). But this *in vitro* data is irrelevant with regard to the presently-claimed method.

The Examiner relies on the disclosure in Example 3 of Nitsche that increase in plasma endotoxin activity was reduced by approximately 58.5% in comparison to an albumin control group by administering Lf. However, the Examiner has ignored the critical fact that the *in vivo* testing in Example 3 involves the use of **Lfs bound to metal ions**, not Lf *per se*.

As previously pointed out, the mechanisms of inflammation and immunity in the living body are very complicated, as previously applied, and now withdrawn, prior art, i.e., *Infection and Immunity*, Vol. 66, No. 2, pp. 486-491, February 1998 (Elass-Rochard et al) states:

> Further in vivo studies are needed to investigate whether Lf could directly overcome the LBP-medicated activation of cells in the host and modulate the CD14-independent LPS signal-pathways.

Accordingly, the *in vitro* tests of Nitsche cannot be predictive or otherwise suggest the presently-claimed invention.

In addition, the Examiner has apparently misunderstood the description in Example 3 with regard to reduction of albumin concentration in blood. Rather, Example 3 shows that when animals in the control group received a corresponding dosage of albumin, rather than the iron bound Lf, endotoxin activity increased. Thus, Example 3 does **not** show reduction of albumin concentration in the blood of the animals of the control group, nor does it show that Lf can alleviate the decrease of albumin concentration in blood. Moreover, Example 3 shows the biochemical action that endotoxemia was induced by increasing of endotoxin amount transferred into the blood, and does not show inflammation. Endotoxemia is a state of

endotoxin present in the blood. Endotoxemia is not inflammation, nor is it a symptom of inflammation.

The Examiner relies on the description herein at paragraph (0004) that "[i]n sepsis caused by gram-negative bacilli, it is known that decline in blood albumin concentration, decrease of lympocytic leukocytes, and increase of neutrophils occur," and that Applicants have acknowledged that "bovine-type lactoferrin has been used to demonstrate an effect of alleviating various symptoms, which appear after infection." The Examiner concludes therefrom that "albumin exudation or increase of blood neutrophils at the inflammatory site, these are expected natural occurrence during inflammation whatever the cause of inflammation is."

In reply, the Examiner ignores the description at the last three lines of paragraph (0005) that it has never been examined whether Lf can be used to alleviate the exudation of plasma albumin at the inflammatory site or the increase of blood neutrophils. Moreover, Applicants have distinguished between hLf and, for example, animal, such as bovine-type, Lf. Indeed, as demonstrated in Example 2 herein, hLf suppresses accumulation of albumin to a significantly greater extent than bovine-type Lf. Indeed, this is further evidence that alleviating a symptom from LPS-induced inflammation is different from treating endotoxemia, since the only distinction Nitsche makes between hLf and animal Lf is its mode of administration. As discussed above, Nitsche discloses: "Lf used for parenteral applications must be equivalent to human Lf, while Lf from animal sources can be used in enteral applications" (column 4, lines 22-25).

The Examiner finds that Nitsche discloses the administration of Lf at a dosage of 0.1 mg/g and 300 mg/kg to inhibit endotoxins, relying on the Abstract and Example 4.

In reply, 0.1 mg is an amount of iron, **not** an amount of Lf; 300 mg is the amount of a solution of bovine-type Lf, **not** the amount of a solution of hLf. In addition, the bovine-type

Lf is one bound to iron ions. Moreover, Example 4, like Example 3, shows only endotoxemia, **not** inflammation or an inflammation symptom, and the Lf used shows suppressing increase of endotoxin concentration, as in Example 3.

It is understood that the *in vitro* and *in vivo* data in Nitsche show only the ability of Lfs to maintain or support phagocytic activity of neutrophils. The phagocytic activity of neutrophils usually decreases depending on the amount of LPS administered and the time lapse after the administration thereof. However, its activity is maintained by the administration of Lf; this fact was reported in M. Yajima et al, FASEB JOURNAL, 13(4), A591 (1999) and Masako Yajima et al, The 4th International Conference on Lactoferrin, Program & Abstract, pp. 77 (1999), a copy of each being **submitted herewith** as part of an Information Disclosure Statement (IDS). When bacteria are eaten by neutrophils, endotoxin is not released, thereby the increase in endotoxin concentration in blood being suppressed as shown in Nitsche.

Example 4 herein demonstrates that administering hLf 18 hours before administration of LPS is much more effective in suppressing the accumulation of albumin than the administration 15 minutes before or 60 minutes after administration of LPS. These results show that the suppressing action of the present invention is different from the mechanisms at work in Nitsche, wherein the time of Lf administration is near the time of bacteria inoculation and the time of antibiotic administration.

In sum, Nitsche neither anticipates nor otherwise suggests the presently-claimed invention. Accordingly, it is respectfully requested that the rejections over Nitsche be withdrawn.

Applicants respectfully call the Examiner's attention to the above-discussed IDS **submitted herewith**. The Examiner is respectfully requested to initial the Form PTO 1449 submitted therewith, and include a copy thereof with the next Office communication.

All of the presently-pending claims in this application are now believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

Respectfully submitted,

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